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B. anthracis in Colloid Sacs in the Rabbit Organism

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As early as 1891-1893 Sanareli stated that virulent anthrax bacilli enclosed in colloid sacs and submerged in the peritoneal cavity of a rabbit will gradually decrease in virulence. The more comprehensive experiments of Sanareli and Alessandrini (1933) in this direction convinced them that under the influence of the humoral factors of the animal organism a deep dissociation process also occurs in parallel with the decrease of virulence in the B. anthracis culture.

Thus, in the experiments of Sanareli and Alessandrini the virulent B. anthracis strain No. 89, which kills a rabbit within 36-48 hours, significantly decreased in virulence after a 34-day retention in the peritoneal cavity of this animal and killed a rabbit only after 60 days. This same virus, after having been in a rabbit's peritoneal cavity for 50-60 days, was not lethal to rabbits, but killed guinea pigs and mice within 4 days. A virus that had been in a rabbit's peritoneal cavity for 83 days lost completely its virulence for all three of the indicated species of animals.

The causes of the decrease in virulence and the change of the basic biomorphological properties of B. anthracis in the rabbit organism evidently must be the prolonged influence of the animal's body heat and the bactericidal effect of the juices and fluids of its organism. In the infection of a rabbit by a highly virulent strain of B. anthracis the defense properties of the organism's mesenchymal cells are paralyzed by the effect of the aggressins produced by the anthrax bacilli. With the enclosure of the anthrax bacilli in colloid sacs and a prolonged presence of these sacs in a rabbit's peritoneal cavity there occurs a gradual weakening of the virulence of the bacilli situated in the sacs. If one should even suppose that the aggressins could, in part, penetrate the walls of the colloid sacs, then in this case they could only be a beginning of immunization because in a natural infection the aggressins are the factor that leads the infection by corroding the cells of the organism. But the microbe that has been localized and enclosed from all sides is subject to the effect of factors that are only harmful to it. As Sanareli and Alessandrini maintain, the anthrax bacillus (in a sac) is subjected to

the bactericidal action of the rabbit's peritoneal lymph; this was also established by them experimentally, by tests in vitro.

Thus, it is possible to produce various degrees of virulence in B. anthracis by the colloid-sac method. The newly acquired properties are not of a temporary, transient character, but on the contrary, as Sanareli and Alessandrini emphasize, become "fixed," transferable in an innumerable amount of transplants and passages on animals. Fabiya, in the Sanareli Institute, in addition to the different degree of virulence of B. anthracis, produced dissociation of this microbe on the R-, Rs-, and S- variants by this method.

We conducted similar experiments, but modified the method as follows. Sacs containing viruses of varied virulence were submerged or sewed into the peritoneal cavity of the rabbit, whereupon, there was always a sac among them containing a culture of a standard anthrax virus in a dose of 0.01 cm³ of broth culture, which had killed a control rabbit in a period of 40-48 hours. The sacs were submerged in the peritoneal cavity or sewed into the omentum after a preliminary laparotomy. A three-stage suture was put on the wound. The sacs were left in the rabbit's peritoneal cavity for various periods of time: 15; 30; 60; 90; 120; 150; 180; and 210 days. At the indicated time periods we again conducted a laparotomy, extracted the sacs, subjected their contents to direct microscopy and then planted them on agar and broth (pH 7.4) for a study of the cultures and the determination of their virulence. The results of our experiments can be reduced to the following.

1. We have established a dissociation of all of the virulent and slightly virulent strains of B. anthracis that had stayed in the colloid sacs even 15 days (in the rabbit's peritoneal cavity), tending towards R-, Rs, rS, S and Sm.
2. A protracted stay of B. anthracis (under the indicated conditions) in the rabbit organism leads to the gradual decrease and complete loss of virulence in all the strains, including the highly virulent standard strain.
3. The decline of virulence of B. anthracis, which occurs from the effect of residence in colloid sacs in an animal organism, is accompanied with deep processes of dissociation and with the appearance of various adaptation forms. These adaptation forms are expressed in the differentiation of the chromatin substance into larger complexes that disengage in the form of round and oval corpuscles with sizes of 0.8-1.5 μ . These corpuscles give a positive reaction to the Fuelgen and Rosenbeck thymonucleic test and are stained to a strawberry or red color according to Giemsa.
4. The pyriform and spherical bodies with a size from 2 to 7-8 μ that appear in the indicated B. anthracis cultures must be considered as different adaptation forms. In staining these corpuscles by the Giemsa method their periphery takes on a blue color; the round or oval body or the group of granules situated inside them are stained a strawberry color. These granules give a positive Fuelgen reaction.
5. Upon transplant of the "labile" B. anthracis cultures, which have been long situated in a rabbit organism, onto fresh nutrient media the adaptation

forms disappear, but appear again by the 5th-7th day of growth. A similar manifestation with particular regularity has been shown in agar cultures.